

Appl. No. 10/731,741

Response dated April 22, 2005

Reply to Office action of January 25, 2005

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently amended) An *in vitro* system comprising a cell preparation comprising OP9 stromal cells that express a Notch ligand that supports T cell lymphopoiesis but does not support B cell lymphopoiesis, wherein the Notch ligand is Delta-like-1 or Delta-like-4.

2. (Currently amended) An *in vitro* system of claim 1 ~~comprising a~~ wherein the Notch ligand ~~that~~ induces T cell lineage commitment and differentiation, stage-specific progenitor expansion, TCR gene rearrangement, and T cell differentiation by hematopoietic progenitors and embryonic stem cells in the absence of the thymus.

3. (Canceled)

4. (Currently amended) An *in vitro* system of claim ~~3~~ 1 that induces TCR V(D)J rearrangement, and T cell differentiation by hematopoietic progenitor cells or embryonic stem cells.

Claims 5 - 7 (Canceled)

8. (Currently amended) An *in vitro* system as claims in claim ~~3~~ 1 wherein the cells lack functional macrophage colony stimulating factor (M-CSF).

9. (Canceled)

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10. (Currently amended) An *in vitro* system as claimed in claim 9 1 wherein the OP9 cells comprise a Delta-like-1 nucleic acid sequence shown in SEQ ID NO:8 or SEQ ID NO:9.

11. (Currently amended) An *in vitro* system as claimed in claim 9 1 wherein the OP9 cells comprise a Delta-like-4 nucleic acid sequence shown in SEQ ID NO:10 or SEQ ID NO:11.

12. (Currently amended) A method of forming cells of the T cell lineage comprising culturing stem cells or progenitor cells that are capable of differentiating into cells of the T cell lineage with an *in vitro* system of claim 1 to form cells of the T cell lineage.

13. (Original) A method according to claim 12 wherein the cells that are capable of differentiating into cells of the T lineage are selected from hematopoietic progenitor cells, hematopoietic stem cells and embryonic stem cells.

14. (Original) A method of claim 12 further comprising separating the cells of the T cell lineage to obtain populations of cells largely consisting of one or more types of cells of the T cell lineage.

15. (Original) A method of claim 14 wherein the population of cells that is separated comprises immature T cells.

16. (Original) A method of claim 14 further comprising inducing the Immature T cells to form mature T cells.

17. (Original) A method of claim 14 wherein the population of cells are formulated in a pharmaceutically acceptable carrier, auxiliary or excipient.

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18. (Withdrawn) A T cell lineage composition comprising cells of the T cell lineage generated with a system as claimed in claim 1 .

19. (Withdrawn) A T cell lineage composition produced by culturing cells capable of differentiating into cells of the T cell lineage with a system of claim 1 and isolating cells of the T cell lineage in the culture.

20. (Withdrawn) A T cell lineage composition of claim 19 comprising one or more of:

- (a) progenitor or precursor cells committed to the T cell lineage;
- (b) CD4⁻ CD8⁻ CD25⁺ CD44⁺;
- (c) cells that have undergone CD4 or CD8 lineage commitment;
- (d) precursor thymocytes that are CD4⁺CD8⁺ double positive (DP);
- (e) single positive cells that are CD4⁺CD8⁺ or CD4⁺CD8⁻ and optionally TCR^{hi};
- (f) TCR- $\alpha\beta$ ⁺ and/or TCR- $\gamma\delta$ ⁺ T cells;
- (g) CD3⁺CD90⁺; and
- (h) mature and functional T cells characterized as TCR/CD3^{high}CD4⁻CD8⁺ or CD4⁺CD8⁻.

21. (Withdrawn) A composition which comprises a nutrient medium that has been conditioned by exposure to a Notch ligand cell preparation that supports T cell lymphopoiesis but does not support B cell lymphopoiesis.

22. (Currently amended) A method for expanding cells of the T cell lineage comprising (a) culturing stem cells or progenitor cells capable of differentiating into cells of the T cell lineage with a system of claim 1; and (b) isolating increased numbers of cells of the T cell lineage.

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23. (Withdrawn) An expanded cellular composition comprising cells of the T cell lineage obtained by a method of claim 22.

24. (Currently amended) A method as claimed in claim 23 ~~22~~ wherein the number of cells is increased by at least about 10 to 15 fold.

25. (Withdrawn) A pharmaceutical composition comprising cells of the T cell lineage generated with a system of claim 1 and a pharmaceutically acceptable carrier, excipient, or diluent.

26. (Withdrawn) A method for screening for modulators of cells of the T cell lineage comprising the steps of:

(a) generating cells of the T cell lineage with a system as claimed in claim 1 in the presence of a test substance; and

(b) detecting the presence or absence of an effect of the test substance on the survival of the cells or on a morphological, functional or physiological characteristic and/or molecular biological property of said cells, whereby an effect altering cell survival, a morphological, functional, or physiological characteristic and/or a molecular biological property of the cells indicates the activity of the test substance.

27. (Withdrawn) A method of treating a patient with a condition involving cells of the T cell lineage or requiring replacement of cells of the T cell lineage comprising transferring a T cell lineage composition as claimed claim 18 into the patient.

28. (Withdrawn) A method according to claim 27 to treat a patient with a T cell deficiency.

29. (New) An *in vitro* system comprising a cell population comprising murine stromal cells that express a Notch ligand that supports murine T cell

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lymphopoiesis but does not support murine B cell lymphopoiesis wherein the Notch ligand is Delta-like-1 or Delta-like-4.

30. (New) An *in vitro* system of claim 29 wherein the Notch ligand induces T cell lineage commitment and differentiation, stage-specific progenitor expansion, TCR gene rearrangement, and T cell differentiation by hematopoietic progenitors and embryonic stem cells in the absence of the thymus.

31. (New) An *in vitro* system of claim 29 that induces TCR V(D)J rearrangement, and T cell differentiation by hematopoietic progenitor cells or embryonic stem cells.

32. (New) An *in vitro* system as claimed in claim 29 wherein the cells lack functional macrophage colony stimulating factor (M-CSF).

33. (New) An *in vitro* system as claimed in claim 29 wherein the stromal cells are OP9 cells.

34. (New) An *in vitro* system as claimed in claim 33 wherein the OP9 cells comprise a Delta-like-1 nucleic acid sequence shown in SEQ ID NO:8 or SEQ ID NO:9.

35. (New) An *in vitro* system as claimed in claim 33 wherein the OP9 cells comprise a Delta-like-4 nucleic acid sequence shown in SEQ ID NO:10 or SEQ ID NO:11.

36. (New) A method of forming cells of the T cell lineage comprising culturing murine stem cells or progenitor cells that are capable of differentiating into cells of the T cell lineage with an *in vitro* system of claim 29 to form cells of the T cell lineage.

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37. (New) A method according to claim 36 wherein the cells that are capable of differentiating into cells of the T lineage are selected from hematopoietic progenitor cells, hematopoietic stem cells and embryonic stem cells.

38. (New) A method of claim 36 further comprising separating the cells of the T cell lineage to obtain populations of cells largely consisting of one or more types of cells of the T cell lineage.

39. (New) A method of claim 38 wherein the population of cells that is separated comprises immature T cells.

40. (New) A method of claim 38 further comprising inducing the immature T cells to form mature T cells.

41. (New) A method of claim 38 wherein the population of cells are formulated in a pharmaceutically acceptable carrier, auxillary or excipient.

42. (New) A method for expanding cells of the T cell lineage comprising (a) culturing murine stem cells or progenitor cells capable of differentiating into cells of the T cell lineage with a system of claim 29; and (b) isolating increased numbers of cells of the T cell lineage.

43. (New) A method as claimed in claim 42 wherein the number of cells is increased by at least about 10 to 15 fold.